

## 15. CONTROL OF THE MOSAIC DISEASE OF TOMATO BY SEEDLING INOCULATION WITH THE ATTENUATED STRAIN OF TMV

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The mosaic disease of tomato caused by TMV is one of the most serious diseases of tomato especially in plastic greenhouse culture in Japan. In order to prevent the disease, seed disinfection, fumigation of seedbed soil, and some cautions against the mechanical contamination are partly evaluated, but these methods are not so effective to prevent the mosaic disease in the successive commercial tomato cropping of the cultivars susceptible to TMV.

In 1963 Oshima et al. (1965) obtained good results in the control of the mosaic disease by inoculation with their attenuated strain of TMV designated as L 11. Komochi et al. (1966) also obtained higher total yields from fieldgrown tomato plants inoculated with the L 11.

Subsequently at both Chiba and Shizuoka Prefectural Agricultural Experiment Stations, the effectiveness of the attenuated strain against the mosaic disease was confirmed in the examination for its practical application (Aoki and Ogiwara, 1974).

It is well known that the mild strain of a virus has been successfully used as the vaccine for preventing various human and animal diseases, but it has been hardly applied practically to plant diseases except in one or two instances in Europe (Rast, 1975; Fletcher and Rowe, 1975). As it has become evident that the attenuated strain of TMV is effective for the control of mosaic disease of tomato caused by TMV, practical application of the strain is earnestly desired by many tomato growers.

This paper presents a review of the practical application of the strain for the mosaic disease control performed at the Chiba Prefectural Agricultural Experiment Station as part of the joint investigation with the Institute for Plant Virus Research and also includes a brief report of the investigation progress in Japan.

### **Multiplication of the attenuated strain of TMV**

The attenuated strain of TMV used at present in Japan is Goto and other's L 11A which was improved in 1968 by repeatedly inoculating with Oshima and other's L 11 on both tomato plants and *Nicotiana glutinosa*. The L 11A is very stable, and it induces almost no visible symptoms on tomato plants after inoculation and scarcely becomes virulent in its successive use (Goto and Nemoto, 1971).

At the Chiba Prefectural Agricultural Experiment Station, tomato seeds treated with dry-heat at 70°C for 3 days are sown in rows 6 cm apart on carbonized rice-hulls in seedbeds in greenhouse for the purpose of multiplication of L 11A.

The tomato leaf sap infected with L 11A is diluted 50 times in concentrations by sterilized water containing 20 g carborundum 600 mesh per L and the inoculum is sprayed powerfully onto tomato seedlings in the stage of 1-2 true leaves at a distance of 3-5 cm by a manual sprayer. Each of these seedlings is transplanted into a 10 cm diameter

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plastic pot with the carbonized rice-hulls in the stage of 3 leaves.

The careful assays for viruses should be repeated at least three times before harvest of the leaves. At first, in order to confirm the infection of L 11A before transplanting, top leaves of the seedlings are picked up at random and inoculated individually on *N. glutinosa* or *N. tabacum* cv. Xanthi-nc.

Second, after transplanting, in order to check other contaminated virulent virus infection inoculation tests of the seedlings are conducted every 10 days on *N. glutinosa*, *Chenopodium amaranticolor*, tomato plant and others. If a seedling showing mosaic symptom is found, it should be removed at once.

Only symptomless leaves of the seedlings are cut off with scissors in the stage of 8–10 leaves in 50–60 days after seedling, and packed in a vinyl bag to preserve them in a freezer below  $-20^{\circ}\text{C}$ . One to 1.2 kg young tomato leaves are harvested per  $\text{m}^2$  seedling bed. More yields of L 11A would be obtained from older tomato plants, but they may be infected with contaminated viruses during the long time culture.

Although the yields of L 11A may be slightly variable with the cultivars of tomato, any cultivars susceptible to TMV will do for the multiplication of L 11A. The cultivar Fukuju No. 2 is used in Chiba Prefecture following the Institute for Plant Virus Research.

### **Tomato cultivars and multiplication of L 11A**

The yields of L 11A did not conspicuously vary among susceptible cultivars of tomato but very little multiplication of L 11A occurred in TMV-resistant varieties. The resistant cultivar, such as Raiden, which has TMV-resistant gene Tm showed low percentage infection and poor yields of L 11A. Similar results were obtained in another group of resistant varieties which have another TMV-resistant gene Tm-2<sup>a</sup>.

Both breeding lines of GCR-237 and GCR-267 which are homogeneous with the TMV-resistant genes were rather less susceptible to L 11A than cultivar Raiden (Ogiwara and Aoki, 1976). The resistant cultivar such as Ogatazuiko which has a TMV-resistant gene Tm-2<sup>a</sup> sometimes shows necrosis on stem and fruit when infected with TMV, and so some protective methods against TMV will be necessary for the cultivar. But L 11A cannot be applied to Ogatazuiko because it is scarcely susceptible to L 11A, but once infected, it may often result in top necrosis.

### **Effect of L 11A on the mosaic disease of tomato caused by TMV**

Neither severe mosaic symptom nor apparent stunting was observed on the tomato plants (cultivar Fukuju No. 2) which were treated by challenge inoculation with TMV-tomato strain every 10 days after the initial inoculation with L 11A on the tomato seedlings in the stage of one true leaf.

The mosaic disease of tomato by TMV usually causes poor growth as well as fruit set and yield reduction. Although the losses in yield are influenced with the cultivars, culture types and the infection period, the yield reduction ratio is usually 20–30% and sometimes over 50%.

The yield experiment with L 11A was performed in three different cultures of tomato in plastic greenhouse where TMV infection naturally occurred through soil.

L 11A was manually inoculated on the seedlings of tomato cultivar House Homare in the stage of 2–3 leaves. The yield increased by over 50% by inoculating with L 11A in winter crop culture and by about 30% both in early spring and fall crop culture comparing with the yield in each check plot where all the tomato plants were naturally infected with TMV.

Another yield experiment with L 11A for seedling inoculation was performed in the winter crop culture on several cultivars of tomato. The highest yield was obtained in

the cultivar Toko-K which was increased by 3 times in the yields and even the lower yields in the cultivar House Homare showed 50% increase in comparison with each check plot (Ogiwara et al., 1973).

In these experiments, all the tomato plants in check plot showed severe mosaic symptoms during 2 months after transplanting, on the other hand, in the treated tomato plants by seedling inoculation with L 11A, no mosaic symptoms appeared even in 3 months after transplanting.

The practical application of L 11A on tomato is eagerly desired by many growers in the tomato cropping areas with plastic greenhouse where the mosaic by TMV always occurs whenever susceptible cultivars are raised in the successive cropping.

### **Efficient inoculation techniques with L 11A for practical application**

It is apparent the L 11A surely inoculated, even if manually or by spraying, on a healthy tomato plant, shows without failure cross protective ability against natural TMV in several days after inoculation. It is important that the efficient and sure inoculation method of L 11A is established for practical application.

Two methods were examined (Nagai and Takeuchi, 1976); one was the spray inoculation with a small hand sprayer of 400 ml capacity with a pressure-gauge, and the other was the seedlings dipping method L 11A-infected tomato leaf sap.

**1. Seedling age for inoculation** The inoculum of L 11A-infected tomato leaf sap diluted 1:50 with 20 g carborundum 600 mesh per L was powerfully sprayed onto the tomato seedlings at a distance within 5 cm from them with working pressure of 5 kg or more per cm<sup>2</sup>. The diluted inoculum of 0.5 L leaf sap was enough to inoculate about 1,000 seedlings in 1 m<sup>2</sup> seedbed.

Results of inoculation test indicated that the symptomless infections which were assayed by *N. glutinosa* were always taken place on the seedlings whenever inoculated in the stage from cotyledon to 3 true leaves. The best age of seedling for inoculation was considered to be in the stage of 1-2 true leaves because the growth of tomato plants was better in early inoculation than in late inoculation immediately before transplanting and also early inoculation caused them cross protective ability from earlier stage.

**2. Concentrations of the inoculum** It is apparent that L 11A is infective on tomato seedlings by manual inoculation with the leaf sap diluted 1:100,000. However, spray inoculation at a distance of 2-3 cm, in the working pressure of 5 kg per cm<sup>2</sup> and by the amount of 0.5 L per m<sup>2</sup> seedbed indicated that 100% infection was guaranteed by 1:25-1:100 in concentrations of the inoculum, 80% infection was observed by 1:200, and only 20% by 1:500. But brushing immediately after spray on the seedlings by a bundle of such as rice culms guaranteed 100% infection even by 1:200 in concentrations of the inoculum.

**3. Quantity of the inoculum** Spraying experiments with L 11A as diluted leaf sap on tomato seedlings by the quantity of 0.25-1.0 L per m<sup>2</sup> indicated that 0.5-1.0 L of the inoculum per m<sup>2</sup> was essential to 100% infection of seedlings and 0.25 L was too little for successful inoculation, however, 100% infection occurred when additional brushing inoculation was made. But the brushing technique is not profitable because of the possibility of inoculating virulent strain of TMV as well as L 11A on the seedlings.

**4. Pressure of sprayer and spraying distance to seedlings** Under the working pressure of 5 kg per cm<sup>2</sup> of the sprayer at a distance of 2.5-5 cm to the seedlings, L 11A was successfully inoculated on the seedlings by an ordinary little sprayer, but the infection ratio is remarkably reduced by spraying at a distance of more than 10 cm to the seedlings. It is necessary for successful inoculation that spraying is conducted at a distance as near as possible.

In the spray inoculation of L 11A mentioned, the temperature desirable is 25-30°C

in the daytime and approximately 20–22°C at night.

**5. Dipping method for the inoculation with L 11A** A bundle of about 20 tomato seedlings in the stage of 2–3 leaves were dipped into the L 11A-infected tomato leaf sap diluted 1:100–1:800 with 20 g carborundum 600 mesh per L and shaken for about 10 seconds. Immediately after the dipping, each seedling was transplanted in a 10 cm diameter plastic pot. It was proved that the plots were successfully infected with L 11A in 10 days later.

Although this technique is easy and performed as efficiently as the spray inoculation with comparatively less inoculum, the risk of infection with virulent TMV as well as L 11A is reasonably suggested in this technique as in the spray inoculation with brushing technique.

These suggestions in brushing and dipping methods proved to be true in another experiment (Nagai and Takeuchi, not presented). Tomato seedlings infected 5% with TMV in the stage of cotyledon became infected more than 90% in the stage of 6–7 leaves because of the mechanical contamination with virulent TMV in the brushing and the dipping process.

On the other hand, tomato seedlings infected 5% with TMV in the stage of cotyledon did not extend more than the initial infected rate even in the stage of 6–7 leaves when successfully spray inoculation was conducted with L 11A in the stage of cotyledon.

### Instructions concerning practical application of L 11A

Tomato seeds must be disinfected either with dry-heat treatment at 70°C for 3 days or with 10% Na<sub>2</sub>PO<sub>4</sub> solution for 20 minutes. Of course, workers should wash their hands thoroughly with soap just before inoculating with L 11A or transplanting of tomato seedlings.

If plenty of the attenuated strain L 11A is prepared, spray inoculation is desirable and it should be required to spray as powerfully as possible at a possibly near distance. Moreover it should be remembered that L 11A in leaf sap unexpectedly reduces the infectivity in a week or so under the room temperature quickly and so it should be used as early as possible if it is prepared as the leaf sap even if stored in an ice box.

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### Discussion

**D. A. Benigno**, Philippines: (1) How did you attenuate TMV for use in the inoculation of tomato?

(2) Is it possible or recommendable for other plant virologists from other countries to import L 11A virus for use in their countries to control TMV on tomato?

**Answer:** (1) The attenuated TMV was firstly produced by Dr. Oshima and his co-researcher, and Dr. Goto and his co-researcher improved it. They attenuated TMV by the following method. First, TMV was inoculated on some pieces of tomato stems and then incubated at 35°C for 2 weeks, thereafter. The attenuated mutants produced during the incubation were selected by means of repeated single lesion isolation from inoculated (*N. glutinosa*) leaves to virus free tomato seedlings.

(2) It is possible to import L 11A from the National Institute for Plant Virus Research. But it is not always recommendable unless very careful instructions are provided in its use.

**K. C. Ling**, IRRI: Is there no mutation of attenuated strain of TMV?

**Answer:** There are some other promising mutants of attenuated strain of TMV which are different from L 11A in the laboratory tests. We always check harmful mutation in a process of multiplication of L 11A, but fortunately L 11A is so stable that it did not mutate in these several years.

**E. W. Kitajima**, Brazil (Comment): L 11A virus is rather stable, Dr. Oshima said. Here, I would like to say that citrus stem-pitting virus is comparatively stable and useful in practical use, too.

**Soelaeman Tirtawidjaja**, Indonesia: TMV affects many species of plants. In connection with situation in my country is there any danger of attenuated strain of TMV is harmful to other species like tobacco?

**Answer:** Goto and Nemoto (1971) inoculated L 11A to 94 species belonging to 20 families and found that 54 species from 15 families were susceptible to L 11A, but the majority of tested species infected systemically showed no symptom or only very mild symptom except in the 2 cases of *Petunia hybrida* and *Physalis floridana* which showed severe symptom.

One of oriental type of tobacco cv. Samsun showed very mild mottling due to the infection of L 11A. Tested bright type tobacco (cv. Bright Yellow) and white burley type tobacco which are resistant to the tomato strain of TMV under field condition, showed necrotic local lesions on leaves inoculated with L 11A.

It is considered that L 11A is not harmful to any other important crops as well as tomato so far as it not change to the worse.